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Familial Spastic Paraparesis: Evaluation of Locus Heterogeneity, Anticipation, and Haplotype Mapping of the SPG4 Locus on the Short Arm of Chromosome 2

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Familial spastic paraparesis (SPG) is a clinically and genetically heterogeneous group of disorders. At least three loci have been implicated in autosomal dominant pure SPG and mutations in either of two loci may cause the X-linked form. Although the penetrance is high for all forms by age 60, there is wide variation in clinical characteristics, including age of onset. Two-point and multipoint linkage analyses in nine families provided supportive evidence that the most common form of SPG is linked to chromosome 2 (SPG4). Haplotype analysis localized the critical region to a 6 cM interval between D2S392 and D2S367. By haplotype analysis, the disease in at least one family does not appear to be linked to any of the presently known SPG loci, suggesting that there is at least one additional SPG gene. Evaluation of ages of onset in 11 families gave suggestive evidence for anticipation with mean age of onset in parents (41.3 years) being older than mean age of onset in children (26.9 years; $P < 0.005$). ***Am. J. Med. Genet. 74:26–36, 1997.*** **© 1997 Wiley-Liss, Inc.**

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INTRODUCTION

The pure form of SPG is characterized by slowly progressive spasticity of the lower extremities with associated hyperreflexia, extensor plantar responses, and the absence of additional features such as mental retardation, peripheral neuropathy, or retinopathy seen in the complicated forms [Sutherland, 1975: McKusick, 1990]. Autosomal dominant, autosomal recessive, and X-linked recessive pure SPG have been reported. Both the X-linked and autosomal forms are genetically heterogeneous. One subset of X-linked spastic paraplegia is allelic to Pelizaeus-Merzbacher disease [Saugier-Veber et al., 1994; Cambi et al., 1996]. A locus on chromosome 8 appears to be involved in one autosomal recessive form of SPG [Hentati et al., 1994a], whereas SPG3, SPG4, and SPG6 loci on chromosomes 14q [Hazan et al., 1993], 2p [Hentati et al., 1994b], and 15q [Fink et al., 1995], respectively, have been implicated in the autosomal dominant form. Nine multigenerational families were studied here to investigate the relative frequencies of the subtypes and the gene localizations. In recent years, expansion of trinucleotide repeat sequences in the relevant genes has been found to be the etiologic mechanism for many neurologic disorders, including Huntington's disease and dominant hereditary ataxias [reviewed in Sutherland and Richards, 1995]. The instability in the expanded sequence leads to further enlargement of the sequence length in subsequent meioses, resulting in increased severity and earlier onset of disease in successive generations. Because of the apparent propensity for this mechanism to be operative in neurologic disorders, we evaluated age of onset in families with SPG for evidence of anticipation.

MATERIALS AND METHODS

Clinical History

Partial pedigrees of nine multigenerational families with SPG (FSP01, FSP02, FSP03, FSP04, FSP06,

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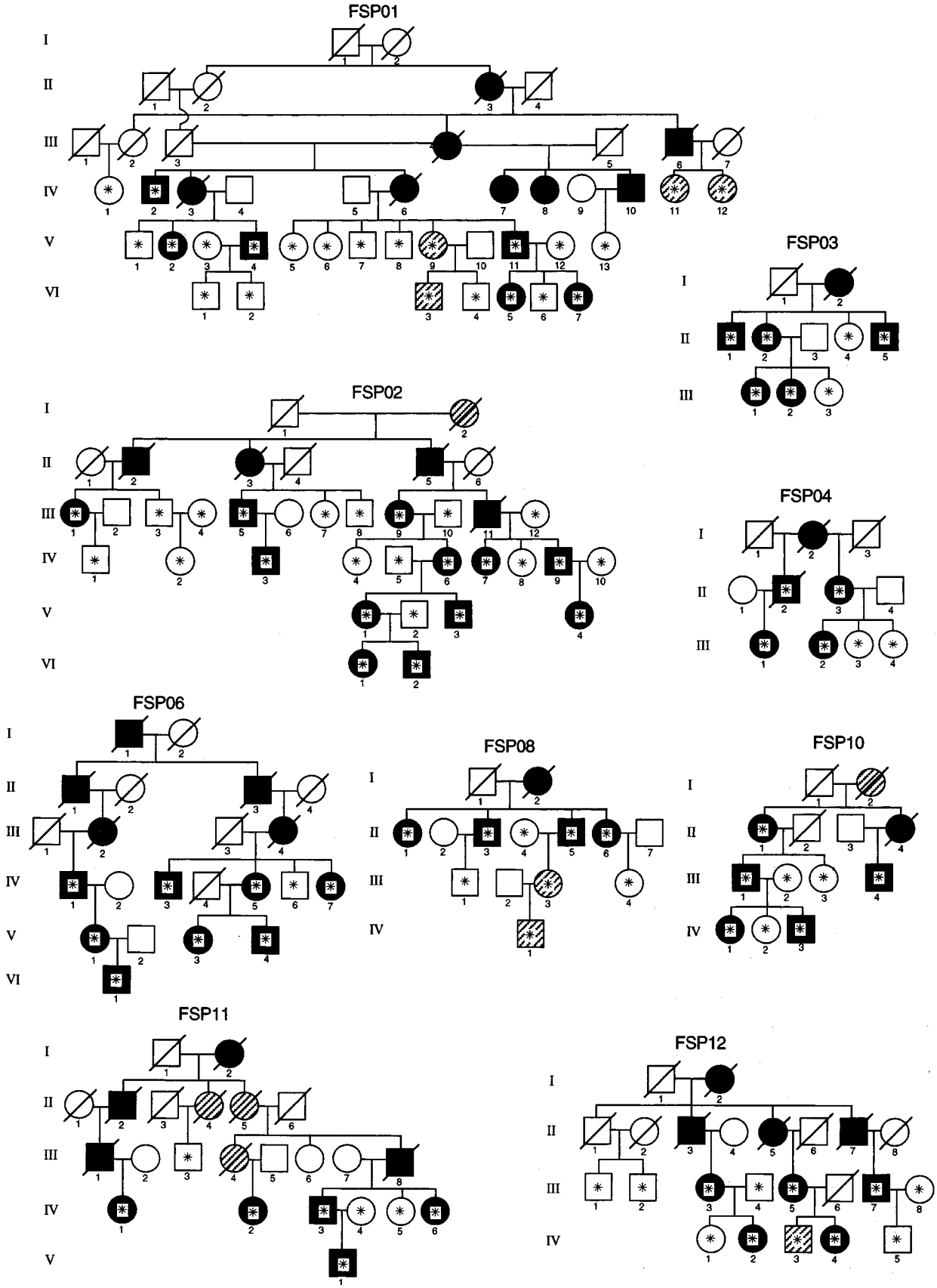

Fig. 1. Partial pedigrees of nine multigenerational families with familial spastic paraparesis. Black symbols denote affected individuals; hatched symbols denote individuals in whom the diagnosis is suspected but uncertain. Samples were obtained from individuals identified by an asterisk.

FSP08, FSP10, FSP11, and FSP12) are shown in Figure 1. These pedigrees depict sampled and examined family members and their pertinent relatives. All probands had bilateral hyperreflexia and Babinski responses documented by a neurologist (TDB). No family member had mental retardation, peripheral neuropathy, retinopathy, or other signs of complicated SPG. The possibility of misassigning disease status was minimized by obtaining samples only from individuals who were clinically evaluated by us or by another neurological specialist or whose medical records documented the diagnosis. Samples were obtained from 110 individuals, 57 of whom were documented as affected.

Evaluation for Anticipation

Because of suggestive evidence of X-linkage, family FSP01 was excluded from the analysis for anticipation. Three additional families with pure SPG that were too small for linkage analysis were included, for a total of 11 families analyzed for anticipation. For each individual evaluated, the age of onset was considered to be the age at which the person and another family member agreed that he or she first noted difficulty walking or running in comparison with peers. Asymptomatic individuals diagnosed with SPG by physical exam only were not assigned an age of onset. If age of onset was known for both generations, the mean age of onset was calculated for the parents and children and the differences were analyzed with a two-tailed Student's t-test.

DNA Analyses

Under protocols approved by the Institutional Review Board of the University of Washington, blood samples were obtained from individuals marked by an asterisk (Fig. 1). DNA was extracted from leukocytes or cell lines and PCR-amplified as previously described [Raskind et al., 1995]. One primer of each pair was end-labeled with [γ^{32}]P by a T4 kinase reaction. In some cases, multiplex PCR reactions with two to three primer sets were done when the range of allele sizes allowed clear separation of products. Primers for the loci D2S174, D2S170, D2S392, D2S390, D2S352, D2S400, D2S367, D2S177, D14S70, D14S69, D14S75, D14S306, D14S269, D14S587, D14S66, D15S122, D15S128, D15S156, D15S165, PLP, DXS52, DXS15, and F8C were obtained from Research Genetics.

Linkage Analysis

Linkage analyses were performed using the MLINK and LINKMAP subprograms of the LINKAGE package version 5.0 [Lathrop et al., 1984] as implemented in FASTLINK (vs) [Schaffer et al., 1994] and using the computer program Vitesse [O'Connell and Weeks, 1995]. Fastlink was used to evaluate linkage in family FSP01, which contained a consanguinous marriage. At present, the Vitesse program cannot analyze complex pedigrees of this nature. Biological relatives of affected individuals were scored as "affected," "at risk" if they were clinically unaffected, or "unknown" if the physical exam was ambiguous. Only biologically unrelated spouses were scored "unaffected" for this rare disease. Autosomal dominant inheritance with a gene frequency of 0.0001 for the disease allele was assumed for the SPG locus.

Calculations were done under two conditions. In one analysis, a probability was assigned to at-risk individuals and all family members were included. Risks were assigned based on age at examination of the at-risk individuals. Because the average age of onset can differ substantially among families, family specific age curves for early, intermediate, and late onset families were constructed based on age stratifications previously suggested by several studies [Harding et al., 1983; Hentati et al., 1994b; Lennon et al., 1995]. The criteria for early onset were onset by age 20 and 96% penetrance by age 20. For families exhibiting intermediate age of onset, a straight line age correction from ages 10–40 was used, with penetrance reaching a maximum of 80% at age 40. Similarly, for families exhibiting a late age of onset, a straight line age correction from ages 10–70 was used, with penetrance reaching a maximum of 80% at age 70. In a second analysis ("low penetrance," "affecteds-only"), phenotypic data were included only on affected individuals, whereas genotypic information was included on all family members. This conservative approach decreases the chance to exclude linkage falsely based on potential crossovers in older "normal" individuals who may actually carry the disease gene but have not expressed it. Confidence intervals for the linkage analyses were determined using the 1-lod-down method [Ott, 1991]. Using these specifications, allowing for heterogeneity (alpha = 0.5) and assuming a four allele system with a polymorphic information content (PIC) of 0.70, 1000 replicates were simulated for each family. Average and maximum lod scores were calculated, as well as the probability for achieving a lod score above 3.0 [Ploughman and Boehnke, 1989].

Allele sizes and frequencies for the marker loci were obtained from the Genome Data Base (GDB). Allele sizes on the autoradiographs were standardized by comparison to DNAs from CEPH family 1331 [Dausset et al., 1990; CEPH]. The order and distance between the marker loci were determined from several linkage maps in the GDB (CEPH sex-averaged linkage maps C2M59, C15M7, C15M25, C14M16, primary linkage average map C14M21, and integrated linkage average map C14M25). The markers that were most informative overall and those that were most likely to flank the SPG3 and SPG4 genes (D2S174, D2S400, D2S367, D2S177, D14S306, D14S269, and D14S587) were used for the multipoint analysis. For computational feasibility, recording of the marker loci to four allele systems for the analysis of family FSP01 using Fastlink was performed as previously described [Speer, 1995].

To evaluate evidence of heterogeneity, admixture analysis (HOMOG) was performed using the two-point lod scores for each autosomal chromosomal location except D2S170, D2S393, and D2S390. For the multipoint analysis, admixture analysis as implemented in HOMOG3R [Ott, 1991] was used. In order to further examine potential heterogeneity, haplotypes were constructed by hand for all tested markers on the four chromosomes to evaluate consistency of linkage assignments.

RESULTS

Pedigree Evaluation

SPG segregated in each of the families with a pattern consistent with autosomal dominant inheritance. In family FSP01, the greater severity and earlier age of onset in males compared with females, the cases of apparent nonpenetrance in females, and the lack of male-to-male transmission suggested that inheritance in this family may be X-linked. There were no definite instances of reduced penetrance in the other eight families. In families FSP02, FSP10, FSP11, and FSP12, instances of male-to-male transmission rules out X-linked inheritance; the remaining pedigrees contain few opportunities to evaluate male-to-male transmission. The mean age of onset, range, and standard deviation for each family is shown in Table I. Families FSP01, FSP03, FSP06, and FSP08 show a late age of onset, with the average age at diagnosis > 40 years and no diagnoses made before age 17. In contrast, all four affected individuals in family FSP10 evidenced symptoms during the first decade. Some members of family FSP11 also were diagnosed in the first decade, but the range was much broader, with an intermediate age of onset distribution. Similar findings were seen in families FSP2, FSP4, and FSP12.

Table II gives the differences between mean age of onset in parents and children in 11 families. There were 34 instances in which the age of onset was earlier in the affected child (range 1–48 years earlier) and only six instances in which the age of onset in the child was equal to or greater than that of the parent (range 0–21 years later). There was no detected relationship of age of onset to sex of parent or child. Mean age of onset in parents was 41.3 ± 18.4 years (n = 25) and mean age of onset in children was 26.9 ± 20.4 years (n = 40) ($P < 0.005$). The affected persons included in this analysis represent 59% of the total persons at risk in each sibship, making it unlikely that the families contain many SPG gene carriers who have not yet expressed the disease. These observations suggest that anticipation may occur in some forms of SPG.

TABLE I. Age of Onset

Family	No. affected individuals studied	Mean age of onset ± SD (range)
FSP01	9	42.7 ± 17.0 (25–72)
FSP02	15	22.7 ± 18.1 (2–53)
FSP03	7	49.0 ± 18.8 (17–75)
FSP04	5	35.8 ± 15.9 (12–52)
FSP06	7	37.1 ± 21 (2–60)
FSP08	6	57.0 ± 13.7 (35–72)
FSP10	4	6.5 ± 3.1 (3–10)
FSP11	7	26.6 ± 16.8 (6–46)
FSP12	6	35.0 ± 12.8 (18–54)

Linkage Analyses

Two-point LOD scores were calculated for seven chromosome 14q markers, five chromosome 2p markers, and four chromosome 15q markers previously found to be linked to SPG3, SPG4, and SPG6, respectively. Simulation studies suggested that four of the nine families (FSP01, FSP02, FSP06, and FSP11) have the potential for a lod score above 2.0 but only FSP01 and FSP02 could produce a lod score above 3.0 (data not shown). Evidence for linkage to chromosome 15 was not obtained in any of the nine families, and close linkage was excluded in FSP1, FSP2, FSP3, FSP6, FSP11, and FSP12 (data not shown). Lod scores for the individual families for markers on chromosomes 2 and 14 obtained with all individuals included in the analysis are given in Table III. To allow for the possibility that the families contain many asymptomatic SPG gene carriers who have not yet expressed the disease, a low penetrance analysis was also done in which phenotypic data were included only on affected individuals, whereas genotypic information was included on all family members. As expected, consistent but lower lod scores were obtained under this assumption (data not shown). Because of the extent of locus heterogeneity, lod scores for the combined families are not presented.

Admixture analysis (HOMOG) using the 2-point lod scores failed to show evidence for heterogeneity in these data ($P > 0.5$). Similar findings were obtained when the multipoint location scores ($\log_{10}$) were examined. Joint analysis of the multipoint location scores for chromosomes 2 and 14 (HOMOG3R) also failed to show evidence for heterogeneity (data not shown). The log likelihood difference between the hypotheses by linkage and homogeneity and linkage and heterogeneity is 0.0. However, it is known from previous studies that SPG is heterogeneous and the patterns of the multipoint analyses for the individual chromosomes suggest there is heterogeneity in our data set as well. Using results of markers on chromosome 2 that flank the critical region for SPG4 [Hazan et al., 1994], a peak multipoint location score of 6.23 was obtained for a position proximal to D2S174 (Fig. 2). This location is outside the critical region for SPG4.

Disease in family FSP02 is very likely linked to SPG4. A maximum lod score of 5.12 at $\theta = 0.00$ was obtained for D2S352 and significant evidence against linkage to chromosome 14 was obtained for multiple markers. Maximum lod scores at $\theta = 0$ of 1.92 for D2S352 and 1.895 for D2S177 for FSP6 and FSP12, respectively, are suggestive evidence for linkage of disease to SPG4 in these families as well. In addition, in both families, the pattern of lod scores makes linkage to chromosome 14 very unlikely and the chromosome 14 haplotypes were discordant (not shown). The individual lod scores for linkage to markers on chromosomes 2 and 14 do not approach significance in families FSP03 and FSP04, and their small size does not allow exclusion of as yet unidentified SPG loci. However, all four affected individuals tested in FSP03 share a chromosome 2 haplotype, whereas the chromosome 14 haplotypes are discordant (data not shown).

TABLE II. Comparison of Mean Age of Onset in Parents and Children

Family	Parent[a]		Child		Difference (yrs)
	Sex	Age of onset	Sex	Age of onset	
FSP02	M	32	F	53	+21
	M	45	M	14	−31
	F	50	F	35	−15
	F	35	F	25	−10
	F	35	M	2	−33
	F	25	F	2	−23
	F	25	M	2	−23
	M	30	F	30	0
	M	30	M	9	−21
	M	9	F	8	−1
FSP03	F	75	M	50	−25
	F	75	F	65	−10
	F	75	M	50	−25
	F	65	F	17	−48
	F	65	F	36	−29
FSP04	M	45	F	42	−3
	F	52	F	28	−24
	F	52	F	12	−40
FSP06	M	50	F	2[a]	−48
	F	60	F	36	−24
	F	60	M	40	−20
FSP08	F	60	F	70	+10
	F	70	F	35	−35
	F	70	F	72	+2
	F	70	M	54	−16
	F	70	M	51	−19
FSP10	F	17	M	5	−12
	M	5	F	8	+3
	M	5	M	3	−2
FSP11	M	42	M	24	−18
	M	42	F	42	0
	M	24	M	17	−7
FSP12	F	39	F	18	−21
	F	54	M	42	−12
FSP14	M	50	M	4	−46
	M	50	F	6	−44
FSP15	F	45	F	20	−25
FSP16	M	35	M	18	−17
	M	18	F	17	−1
	M	18	M	13	−5
		mean 41.3 ± 18.4 n = 25		mean 26.9 ± 20.4 n = 40	$P < .005$

[a] In family FSP06, there is one grandparent-grandchild pair. The parent of the child meets our criteria for FSP but is asymptomatic. Therefore, an age of onset cannot be assigned.

In several families the haplotypes provided suggestive evidence that the disease is not caused by the SPG4 gene. Pertinent portions of three pedigrees are shown in Figure 3a. The haplotypes in FSP08 are inconsistent with linkage to chromosome 2. The genotypes of unsampled individuals I-1 and I-2 were arbitrarily assigned, but reversing the genotypes does not abolish the discordance. No single haplotype is shared by all four affected siblings in generation II. Generally negative lod scores for chromosome 2 markers support this conclusion. The analysis for linkage to SPG3 is inconclusive in this family and would require one individual (II-1) who has hyperactive tendon reflexes and bilateral Babinski responses to be unaffected by SPG, as well as one individual (III-4) to be a nonexpressing carrier for the haplotypes to support linkage (haplotypes not shown). The linkage assignments are uncertain in FSP10 and FSP11, but the patterns of haplotypes are more consistent with linkage of the disease to SPG3 (haplotypes not shown).

Evaluation of family FSP01 is more complex. Two-point analysis provides evidence against close linkage of SPG to chromosome 14 markers when either all individuals or only affected individuals are included (data not shown for the latter analysis). When all individuals are included, a maximum lod score of 2.621 was obtained for D2S174 at $\theta = 0.10$, suggestive lod scores were seen for markers D2S352 and D2S367 and a peak multipoint location score of 2.94 was obtained for a site between D2S400 and D2S367. However, the paternal haplotype of affected individual VI-7 appears to be entirely discordant with that of her affected sister, VI-5 (Fig. 3a). Both sisters have pathologically hyperactive reflexes and take baclofen for flexor spasms of the legs.

TABLE III. Two-point Linkage Analyses for Individual Families

	Recombination fractions						
FSP01	0	0.05	0.1	0.15	0.2	0.3	0.4
D2S174	−∞	2.614	2.621	2.463	2.221	1.574	0.789
D2S352	−∞	2.108	2.087	1.907	1.646	0.971	0.233
D2S400	−∞	−0.556	−0.311	−0.187	−0.112	−0.030	0.002
D2S367	−∞	1.982	1.990	1.864	1.679	1.210	0.648
D2S177	−9.147	1.380	1.681	1.714	1.629	1.257	0.702
D14S70	−∞	−2.071	−1.030	−0.494	−0.185	0.074	0.078
D14S69	−∞	−1.092	−0.574	−0.333	−0.208	−0.102	−0.042
D14S75	−∞	−0.048	0.168	0.242	0.256	0.202	0.106
D14S306	−∞	−3.838	−2.484	−1.727	−1.221	−0.576	−0.197
D14S269	−∞	−0.553	−0.296	−0.167	−0.089	−0.011	0.014
D14S587	−∞	−2.564	−1.599	−1.102	−0.809	−0.464	−0.194
D14S66	−17.247	−3.388	−2.198	−1.535	−1.091	−0.521	−0.184
FSP02							
D2S174	−3.743	−0.313	−0.136	−0.076	−0.057	−0.056	−0.044
D2S352	5.123	4.676	4.207	3.716	3.201	2.106	0.964
D2S400	0.720	0.681	0.611	0.521	0.418	0.204	0.040
D2S367	−∞	1.999	2.006	1.857	1.632	1.057	0.442
D2S177	−∞	0.481	0.954	1.088	1.070	0.795	0.372
D14S70	−∞	1.023	1.073	0.991	0.852	0.502	0.161
D14S69	−8.529	−1.633	−1.005	−0.656	−0.430	−0.169	−0.045
D14S75	−∞	−2.203	−1.123	−0.590	−0.297	−0.064	−0.029
D14S306	−∞	−0.385	0.249	0.491	0.563	0.457	0.215
D14S269	−∞	−3.900	−2.496	−1.714	−1.196	−0.556	−0.198
D14S587	−∞	−2.402	−1.304	−0.736	−0.395	−0.060	0.028
D14S66	−∞	−2.251	−1.158	−0.602	−0.277	0.021	0.072
FSP03							
D2S174	−2.795	−0.059	0.125	0.181	0.184	0.119	0.035
D2S352	0.637	0.562	0.484	0.405	0.325	0.171	0.049
D2S400	−0.606	−0.404	−0.279	−0.193	−0.131	−0.052	−0.012
D2S367	1.353	1.207	1.053	0.892	0.725	0.385	0.103
D2S177	−4.459	−0.982	−0.511	−0.275	−0.139	−0.017	0.006
D14S70	−∞	−0.878	−0.539	−0.348	−0.224	−0.082	−0.018
D14S69	−∞	−0.960	−0.570	−0.358	−0.225	−0.080	−0.017
D14S75	−4.760	−0.324	−0.116	−0.030	0.006	0.019	0.007
D14S306	−∞	−0.806	−0.492	−0.319	−0.206	−0.077	−0.017
D14S269	−0.229	−0.174	−0.126	−0.088	−0.059	−0.022	−0.005
D14S587	−∞	−1.034	−0.609	−0.380	−0.239	−0.080	−0.018
D14S66	−0.389	−0.336	−0.273	−0.212	−0.156	−0.069	−0.017
FSP04							
D2S174	−0.586	−0.377	−0.249	−0.162	−0.100	−0.025	0.005
D2S352	0.001	0.129	0.179	0.192	0.184	0.135	0.069
D2S400	0.230	0.195	0.164	0.136	0.111	0.069	0.034
D2S367	0.226	0.344	0.383	0.381	0.356	0.265	0.142
D2S177	0.217	0.183	0.150	0.120	0.091	0.043	0.011
D14S70	0.968	0.862	0.754	0.647	0.540	0.332	0.145
D14S69	0.659	0.577	0.496	0.418	0.342	0.204	0.089
D14S75	−3.699	−0.721	−0.444	−0.292	−0.194	−0.076	−0.018
D14S306	1.146	1.029	0.909	0.789	0.667	0.424	0.193
D14S269	−3.699	−0.721	−0.444	−0.292	−0.194	−0.076	−0.018
D14S587	−0.075	−0.061	−0.051	−0.045	−0.040	−0.032	−0.021
D14S66	−3.699	−0.721	−0.444	−0.292	−0.194	−0.076	−0.018
FSP06							
D2S174	0.976	0.858	0.737	0.612	0.487	0.245	0.063
D2S352	1.925	1.713	1.499	1.282	1.067	0.652	0.283
D2S400	0.426	0.339	0.261	0.194	0.138	0.059	0.015
D2S367	1.978	1.730	1.484	1.240	1.000	0.545	0.181
D2S177	−∞	0.086	0.271	0.323	0.318	0.219	0.075
D14S70	0.595	0.509	0.425	0.342	0.264	0.127	0.034
D14S69	−0.255	−0.219	−0.180	−0.141	−0.105	−0.047	−0.012
D14S75	−∞	−0.547	−0.251	−0.099	−0.013	0.050	0.031
D14S306	0.294	0.252	0.210	0.169	0.130	0.063	0.017
D14S269	0.191	0.177	0.156	0.131	0.104	0.052	0.014
D14S587	−∞	−1.123	−0.633	−0.376	−0.220	−0.060	−0.007
D14S66	0.251	0.226	0.196	0.163	0.128	0.064	0.017

TABLE III. *(Continued)*

	Recombination fractions						
FSP08	0	0.05	0.1	0.15	0.2	0.3	0.4
D2S174	−0.036	−0.029	−0.023	−0.017	−0.013	−0.006	−0.001
D2S352	−0.236	−0.198	−0.161	−0.126	−0.094	−0.043	−0.011
D2S400	−0.078	−0.072	−0.064	−0.054	−0.044	−0.022	−0.006
D2S367	−3.398	−0.721	−0.443	−0.292	−0.194	−0.076	−0.018
D2S177	−3.398	−0.721	−0.443	−0.292	−0.194	−0.076	−0.018
D14S70	0.301	0.257	0.215	0.173	0.133	0.064	0.017
D14S69	0.185	0.157	0.131	0.105	0.081	0.040	0.011
D14S75	0.301	0.258	0.215	0.173	0.133	0.064	0.017
D14S306	0.330	0.280	0.231	0.182	0.136	0.060	0.013
D14S269	−3.457	−0.768	−0.480	−0.320	−0.214	−0.085	−0.020
D14S587	−3.606	−0.880	−0.564	−0.382	−0.258	−0.103	−0.024
D14S66	−3.606	−0.880	0.564	−0.382	−0.258	−0.103	−0.024
FSP10							
D2S174	1.638	1.474	1.305	1.133	0.957	0.607	0.280
D2S352	0.174	0.146	0.119	0.094	0.072	0.034	0.009
D2S400	−2.515	0.267	0.436	0.478	0.464	0.353	0.185
D2S367	−6.207	−0.534	−0.062	0.154	0.261	0.304	0.205
D2S177	−7.097	−1.358	−0.816	−0.526	−0.341	−0.127	−0.028
D14S70	0.000	−0.423	−0.178	−0.058	0.008	0.064	0.056
D14S69	−2.365	0.369	0.533	0.568	0.548	0.424	0.234
D14S75	−2.938	0.233	0.420	0.477	0.478	0.390	0.225
D14S306	−2.480	0.322	0.494	0.537	0.525	0.413	0.232
D14S269	0.918	0.850	0.776	0.696	0.612	0.432	0.233
D14S587	0.000	−0.928	−0.446	−0.214	−0.084	0.033	0.050
D14S66	0.000	−0.430	−0.181	−0.059	0.010	0.067	0.058
FSP11							
D2S174	−0.304	−0.379	−0.414	−0.386	−0.318	−0.186	−0.096
D2S352	−3.711	−0.374	−0.166	−0.080	−0.040	−0.019	−0.017
D2S400	−2.369	0.871	0.940	0.877	0.756	0.435	0.127
D2S367	−4.908	−0.988	−0.485	−0.226	−0.081	0.039	0.045
D2S177	1.187	1.079	0.969	0.856	0.740	0.500	0.248
D14S70	0.959	0.855	0.742	0.625	0.507	0.280	0.098
D14S69	0.645	0.589	0.526	0.458	0.385	0.232	0.088
D14S75	1.138	0.980	0.821	0.662	0.506	0.216	0.015
D14S306	−3.118	−0.088	−0.002	−0.038	−0.109	−0.216	−0.170
D14S269	1.151	1.048	0.942	0.832	0.717	0.473	0.221
D14S587	−∞	−1.368	−0.703	−0.369	−0.178	−0.005	0.028
D14S66	−0.063	−0.040	−0.029	−0.023	−0.019	−0.012	−0.004
FSP12							
D2S174	−6.673	−0.540	−0.247	−0.103	−0.025	0.033	0.031
D2S352	−0.200	−0.139	−0.077	−0.026	0.010	0.043	0.036
D2S400	−0.667	−0.359	−0.213	−0.128	−0.075	−0.021	−0.002
D2S367	1.218	1.144	1.039	0.912	0.766	0.441	0.144
D2S177	2.128	1.910	1.682	1.445	1.201	0.708	0.279
D14S70	−6.199	−0.136	0.025	0.067	0.066	0.026	−0.003
D14S69	−7.522	−1.031	−0.660	−0.451	−0.315	−0.155	−0.066
D14S75	−∞	−1.327	−0.815	−0.548	−0.378	−0.174	−0.061
D14S306	−6.758	−0.539	−0.295	−0.174	−0.103	−0.031	−0.005
D14S269	−6.764	−0.558	−0.316	−0.195	−0.124	−0.047	−0.012
D14S587	−∞	−1.392	−0.839	−0.551	−0.374	−0.177	−0.073
D14S66	−∞	−1.352	−0.841	−0.576	−0.411	−0.214	−0.095
FSP01							
DXS52	−∞	−7.677	−5.124	−3.668	−2.673	−1.363	−0.537
DXS15	−∞	−3.465	−2.127	−1.493	−1.055	−0.505	−0.171
F8C	−∞	−4.013	−2.409	−1.564	−1.034	−0.435	−0.147

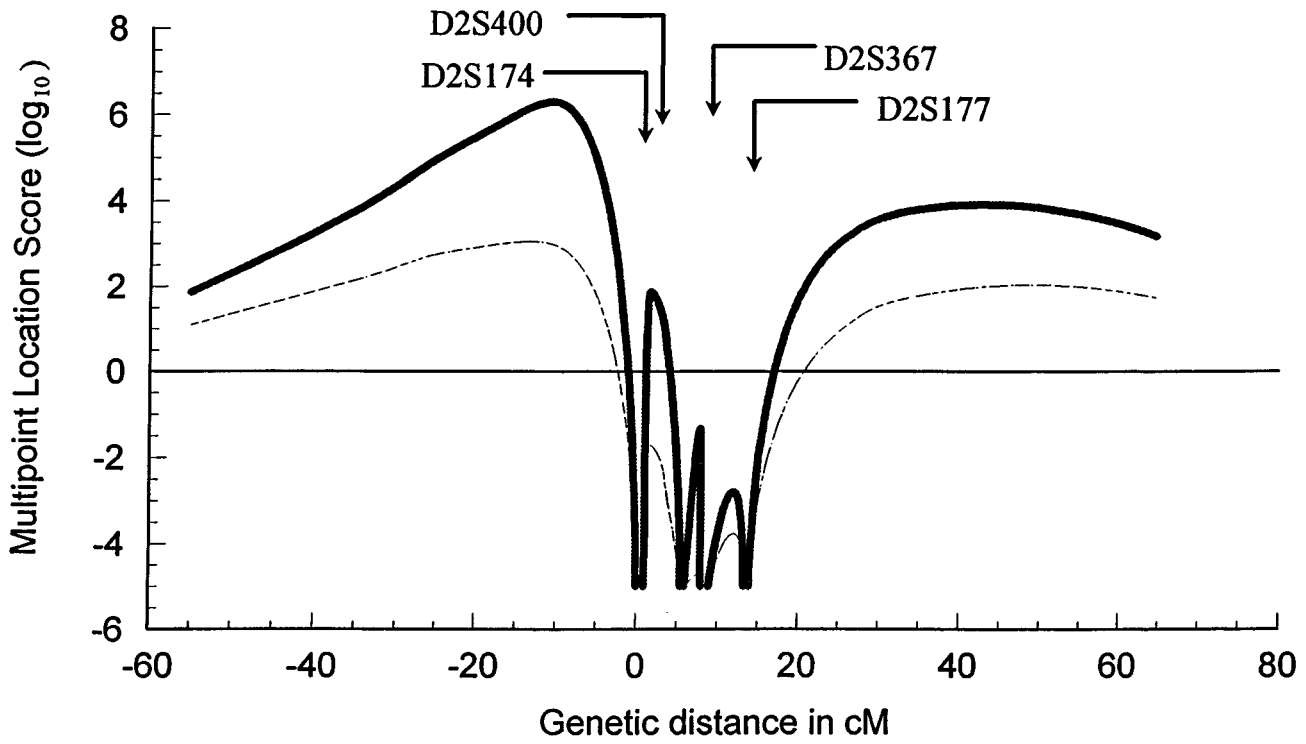

Fig. 2. Multipoint linkage analysis for four markers on chromosome 2p, using the age-specific penetrance curves calculated for each family. The solid line indicates the analysis of all members of the combined families, and the dashed line gives the results for only affected.

It is possible that the affected individuals share a paternal allele at D2S177. However, such concordance would require crossover events between D2S367 and D2S177 in both chromosomes of individual VI-5. Recombination events in other families place the SPG4 gene proximal to D2S367. Disease in family FSP01 may not be linked to any of the identified loci implicated in autosomal dominant SPG.

Chromosome 2 haplotypes in affected individuals in FSP02 were evaluated for recombination events that define the interval containing SPG4. To refine the sites of recombination, additional chromosome 2 markers were genotyped. Haplotypes of a portion of this pedigree are shown in Figure 3. Crossovers in IV-7 and VI-2 place SPG4 in a 6cM interval between D2S367 and D2S392.

Because of the possibility that disease in FSP01 is X-linked, two X chromosome regions previously identified as containing genes for SPG were evaluated. Genotypes for a CA repeat polymorphism in the first intron of PLP did not segregate with the disease (data not shown). Therefore, SPG in this family is not allelic to Pelizaeus Merzbacher disease (SPG2) in Xq21.3-q22 [Saugier-Veber et al., 1994]. Discordant inheritance of SPG and flanking markers tightly linked to L1CAM, a gene in Xq28 involved in SPG1 [Kenwrick et al., 1986; Jouet et al., 1994; Cambi et al., 1996], were observed (Table III; Fig. 3b).

DISCUSSION

In a linkage analyses in nine multigenerational families with spastic paraparesis, we have obtained significant evidence for linkage of SPG to chromosome 2p in one family (FSP02) and suggestive evidence for linkage to this locus in two families (FSP06 and FSP12). Two-point analyses also provided suggestive evidence for linkage of disease in FSP01 to the SPG4 locus and excluded linkage to the SPG3 and SPG6 loci. However, haplotype evaluation of a sibship in generation VI conflicts with this assignment. The pedigree characteristics of FSP01 are compatible with X-linkage, but our data exclude the previously identified SPG1 and SPG2 loci. Haplotype evaluation argues that neither SPG3 nor SPG4 is responsible for disease in FSP08. The findings in FSP01 and FSP08 are consistent with the existence of at least one additional locus for SPG. Mapping results in four of the families (FSP03, FSP04, FSP10, and FSP11) are inconclusive. These data agree with the previous reports that SPG4 is the most common form of SPG and that SPG2 is involved in a smaller subset of families [Hazan et al., 1994; Hentati et al., 1994b; Lennon et al., 1995; Fink et al., 1996]. To date, only a single family with linkage to chromosome 15 has been reported, and many families remain unlinked [Fink et al., 1995, 1996].

Haplotype analysis places the SPG4 locus within the 6 cM interval on chromosome 2p flanked by D2S392 and D2S367. Only a few genes have been mapped to band 2p21 [calmodulin 2 (CALM2), CAD trifunctional protein of pyrimidine biosynthesis (CAD), alobar, or semilobar holoprosencephaly-2 (HPE2, HPC), luteinizing hormone/choriogonadotropin receptor (LHCGR), interferon-inducible, double-stranded, RNA-dependent

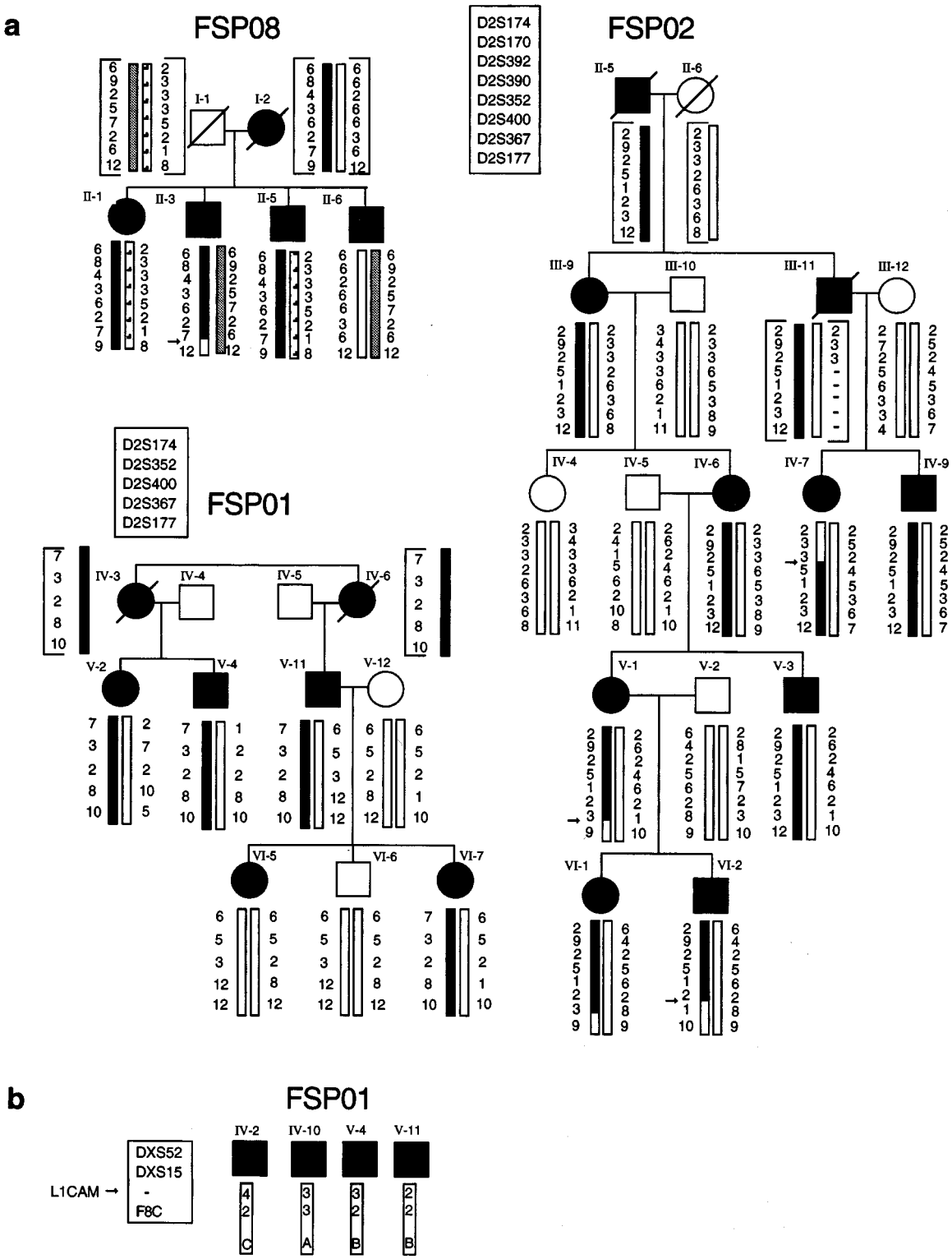

Fig. 3. (**a**) Haplotypes for chromosome 2 markers demonstrating discordance or key recombination events. Relevant portions of pedigrees FSP01, FSP02, and FSP08 are shown. The order of markers given in the insets are according to Genome Data Base map C2M59 (last modified November 7, 1995). Most likely affected chromosomes are shown in black; a shaded segment denotes a possibly affected chromosome portion. Inferred haplotypes are contained within brackets and have been constructed to minimize the number of recombination events. Arrows indicate sites of crossing over. (**b**) Haplotypes of four affected individuals in FSP02 for markers flanking the L1CAM locus on the X chromosome.

protein kinase (PRKR), nonerythrocytic-1 beta spectrin (beta-fodrin; SPTBN1), follicle-stimulating hormone receptor (FSHR, ODG1), and the F, S solute carrier family (SLC3A1)]. CALM is centromeric to the SPG critical region. The positions of the other genes relative to the STRP markers used for linkage mapping are not known, but none has an obvious relationship to SPG from a physiologic or pharmacologic standpoint.

There has been an attempt to divide SPG into two subgroups based on age of onset. Type I SPG is characterized by onset of symptoms before age 35 in most cases, whereas most cases of Type II SPG have onset after age 35 [Harding, 1981, 1983]. It is unclear that this classification is biologically relevant. Five of six type II families studied by Hazan et al. [1994] were linked to chromosome 2, as was a family whose average age of onset was in an intermediate range. This group previously reported linkage to chromosome 14 of SPG in a family whose average age of onset was 6 years (range 2–50 years) [Hazan et al., 1993]. Three late onset SPG families and one early onset family reported by Hentati et al. [1994b] had high likelihood of linkage to chromosome 2, and a family with very early onset (mean age of onset <5 years) showed likelihood of linkage to chromosome 14. Perhaps very early age of onset is associated more often with SPG2. In this study, we were not able to assign linkage for the family with the earliest onset (FSP10).

A disease in which successive generations tend to have earlier onset and increased severity is said to exhibit "anticipation." Instability of intragenic tandemly repeated trinucleotide codons has been found to be the biologic basis of this genetic phenomenon in other neurologic disorders. There may be a systematic bias in ages of onset determined from historical information because subsequent members may recognize their symptoms earlier than the first case in the family. However, our data showing earlier age of onset in 34 of 40 evaluable parent (or grandparent)/child pairs are consistent with anticipation. Other investigators have reported age of onset patterns in a few SPG families that suggest anticipation in both SPG3 [Gispert et al., 1995] and SPG4 [Hazan et al., 1994].

Study of additional families will be needed to determine whether specific SPG genes have distinguishing clinical characteristics, whether anticipation is generally associated with this class of disorders, and to perform more detailed mapping of the loci. SPG is likely to be a disease of layer V pyramidal neurons in primary motor cortex, which presumably utilize glutamate as an excitatory neurotransmitter, GABA as an inhibitory neurotransmitter, and serotonin as both an inhibitory and excititory neurotransmitter [Behan and Maia, 1974; Spain 1994; Van Brederode and Spain, 1995]. Identification of genes involved in SPG will advance our understanding of normal human motor system functioning and suggest possible treatment strategies for the disease.

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